

sequence selected from: LeuCysPheArgMetLysAsp (residues 8-14 of SEQ ID NO:2); ValLeuTyrLeuHisAsn (residues 19-24 of SEQ ID NO:2); GlnLeuLeuAlaGly (residues 26-30 of SEQ ID NO:2); IleSerValValProAsn (residues 43-48 of SEQ ID NO:2); SerProValIleLeuGlyVal (residues 56-62 of SEQ ID NO:2); GlnCysLeuSerCysGlyThr (residues 67-73 of SEQ ID NO:2); ProlleLeuLysLeuGlu (residues 77-82 of SEQ ID NO:2); PheTyrArgArgAspMetGly (residues 101-107 of SEQ ID NO:2); LeuThrSerSerPheGluSer (residues 108-114 of SEQ ID NO:2); PheLeuCysThrSer (residues 121-125 of SEQ ID NO:2); GlnProValArgLeuThr (residues 130-135 of SEQ ID NO:2); PheTyrPheGlnGln (residues 150-154 of SEQ ID NO:2); ArgAlaLeuAspAlaSerLeu (residues 49-55 of SEQ ID NO:2); or GlyLeuHisAlaGluLysVal (residues 31-37 of SEQ ID NO:2); or B) specifically binds polyclonal antibodies generated against a 12 consecutive amino acid segment of SEQ ID NO: 6, 13, or 15; and comprises at least one sequence selected from: SerLeuArgHisValGlnAsp (residues 13-19 of SEQ ID NO:6); ValTrpIleLeuGlnAsn (residues 24-29 of SEQ ID NO:6); IleLeuThrAlaVal (residues 31-35 of SEQ ID NO:6); IleThrLeuLeuProCys (residues 46-51 of SEQ ID NO:6); AspProThrTyrMetGlyVal (residues 63-69 of SEQ ID NO:6); SerCysLeuPheCysThrLys (residues 74-80 of SEQ ID NO:6); ProValLeuGlnLeuGly (residues 85-90 of SEQ ID NO:6); PheTyrHisLysLysSerGly (residues 109-115 of SEQ ID NO:6); ThrThrSerThrPheGluSer (residues 116-122 of SEQ ID NO:6); PhelleAlaValCys (residues 129-133 of SEQ ID NO:6); CysProLeulleLeuThr (residues 138-143 of SEQ ID NO:6); PheGluMetIleVal (residues 154-158 of SEQ ID NO:6); GlnAspLeuSer (residues 18-21 of SEQ ID NO:6); ValProArgLysGluGlnThrVal (residues 35-42 of SEQ ID NO:6); SerLysGlySerCysPro (residues 134-139 of SEQ ID NO:6); ArgAlaAlaSer (residues 8-11 of SEQ ID NO:6); ProCysGlnTyrLeuAspThrLeuGlu (residues 50-58 of SEQ ID NO:6); and SerGlyThrThr (residues 114-117 of SEQ ID NO:6); or ITGTIND (residues 23-29 of SEQ ID NO:13); VWTLQG (residues 34-39 of SEQ ID NO:13); NLVAV (residues 41-45 of SEQ ID NO:13); VAVITC (residues 56-61 of SEQ ID NO:13); DPIYLG (residues 73-79 of SEQ ID NO:13); MCLYCEK (residues 84-90 of SEQ ID NO:13); PTLQLK (residues 95-100 of SEQ ID NO:13); FYRAKTG (residues 119-125 of SEQ ID NO:13); RTSTLES (residues 126-132 of SEQ ID

NO:13); FIASS (residues 139-143 of SEQ ID NO:13); QPIILT (residues 147-152 of SEQ ID NO:13); FELNI (residues 163-167 of SEQ ID NO:13); SMCK (residues 18-21 of SEQ ID NO:13); NDLN (residues 28-31 of SEQ ID NO:13); [VPR(R/S)TSVT] VPRRTSVT (residues 45-51 of SEQ ID NO:13); TCKYPEALE (residues 60-68 of SEQ ID NO:13); TGRT (residues 124-127 of SEQ ID NO:13); or SKGDQP (residues 143-148 of SEQ ID NO:13), or VPRSDSVT (residues 45-52 of SEQ ID NO:15); SKRDQP (residues 143-148 of SEQ ID NO:15). Preferred embodiments include such a polypeptide: wherein the polypeptide comprises a plurality of the described sequences. Preferably the 12 consecutive amino acid segment comes from an IL-18 sequence (see SEQ ID NO: 2):

LeuCysPheArgMetLysAspSerAlaLeuLysValLeuTyrLeuHisAsnAsn (residues 8-25 of SEQ ID NO:2);

IleSerValValProAsnArgAlaLeuAspAlaSerLeuSerProValIleLeuGlyValGln (residues 43-63 of SEQ ID NO:2); SerProValIleLeuGlyValGlnGlyGlySerGlnCys (residues 56-68 of SEQ ID NO:2); ProlleLeuLysLeuGluProValAsnIleMetGluLeu (residues 77-89 of SEQ ID NO:2); ThrSerSerPheGluSerAlaAlaTyrProGlyTrpPhe (residues 109-121 of SEQ ID NO:2); PheLeuCysThrSerProGluAlaAspGlnProVal (residues 121-132 of SEQ ID NO:2); ThrGlnIleProGluAspProAlaTrpAspAlaProlle (residues 135-147 of SEQ ID NO:2); or ThrSerSerPheGluSerAlaAlaTyrProGlyTrpPhe (residues 109-121 of SEQ ID NO:2); or a rodent IL-1ε sequence (see SEQ ID NO: 6):

ArgAlaAlaSerProSerLeuArgHisValGlnAspLeu (residues 8-20 of SEQ ID NO:6);

SerSerArgValTrpIleLeuGlnAsnAsnIleLeu (residues 21-32 of SEQ ID NO:6);

ProValThrIleThrLeuLeuProCysGlnTyrLeu (residues 43-54 of SEQ ID NO:6);

GlyValGlnArgProMetSerCysLeuPheCysThr (residues 68-79 of SEQ ID NO:6);

PheCysThrLysAspGlyGluGlnProValLeuGlnLeu (residues 77-89 of SEQ ID NO:6);

ThrSerThrPheGluSerAlaAlaPheProGlyTrpPhe (residues 117-129 of SEQ ID NO:6);

or CysSerLysGlySerCysProLeuIleLeuThrGln (residues 134-144 of SEQ ID NO:6); or

a primate IL-1ε sequence (see SEQ ID NO: 13 or 15): SMCKPITGTINDL (residues

18-30 of SEQ ID NO:13); NQQVWTLQGQNL (residues 31-42 of SEQ ID NO:13);

PVTAVITCKYP (residues 53-64 SEQ ID NO:13); GIQNPEMCLYCE (residues 78-89

of SEQ ID NO:13); YCEKVGEGPTLQL (residues 87-99 of SEQ ID NO:13);

B<sup>1</sup>  
cont.

TSTLESVAFPDWF (residues 127-139 of SEQ ID NO:13); SKGDQPIILTSE (residues 143-154 of SEQ ID NO:13); SKRDQPIILTSE (residues 143-154 of SEQ ID NO:15); or GKSYNFATFELNIND (residues 156-169 of SEQ ID NO:15).--

Please replace the paragraph on page 13, lines 3-11, and replace with the following rewritten paragraph:

B<sup>2</sup>

-- Figure 1A is a cartoon depicting a top down view through the central axis of the predicted IL-1 $\delta$  or IL-1 $\epsilon$  protein demonstrating the characteristic tertiary  $\beta$ -trefoil structure with its 3-fold symmetric topology. Contact sites of the IL-1 $\delta$  or IL-1 $\epsilon$  protein that are predicted to bind the IL-1 receptor subunits are designated as sites A, B or C (Fig. 2). Contact sites A and C bind to the first receptor subunit of IL-1, while contact site B binds to the IL-1 second receptor subunit.--

Please replace the paragraph on page 16, lines 21-22, and replace with the following rewritten paragraph:

B<sup>3</sup>

--Table 4 shows relationship of IL-1 family members, and Fig. 2 provides an alignment of selected family members.--

Please delete the paragraph (Table 5) on page 24, lines 1-45. ✓

Please add the following new paragraph on page 13, after line 16:

B<sup>4</sup>

--Figs. 2A and B describe polypeptide sequences in the IL-1 family of cytokines. The position numbers refer to alignment, and are not residue numbers from the individual sequences. Various sites for interaction with receptor are: SITE A includes residues corresponding to positions numbered 16-19, 25-27, 32, 34-41, and 44; SITE B includes residues corresponding to positions numbered 9-12, 14, 52-60, 115, 117-118, 122-123, 168, and 170; and SITE C includes residues corresponding to positions numbered 84-109. B conformations correspond to positions 11-17; 22-27; 30-34; 48-53; 65-71; 77-83; 88-93;

$\beta^4$  *incl.*  
110-117; 122-128; 135-139; 145-150; and 168-172. The sequences can be found in the sequence listing, as indicated: hIL-1 $\epsilon$  (SEQ ID NO:15); mIL-1 $\epsilon$  (residues 4-160 of SEQ ID NO:6); mIL-1 $\delta$  (residues 3-160 of SEQ ID NO:2); hIL-1RA (residues 31-177 of SEQ ID NO:7); hIL-1 $\gamma$  (residues 37-193 of SEQ ID NO:8); mIL-1 $\gamma$  (residues 36-192 of SEQ ID NO:9); hIL-1 $\beta$  (residues 117-269 of SEQ ID NO:10); hIL-1 $\alpha$  (residues 127-271 of SEQ ID NO:11)--

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Please replace the paragraph on page 40, lines 16-30, with the following rewritten paragraph:

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$\beta^5$   
--The present invention particularly provides muteins which act as agonists or antagonists of the IL-1 $\delta$  or IL-1 $\epsilon$ . Structural alignment of mouse IL-1 $\delta$  and mouse IL-1 $\epsilon$  with other members of the IL-1 family show conserved features/residues, particularly 12  $\beta$  strands folded into a  $\beta$ -trefoil fold (see Fig 1A; Table 3 and Fig.2A,B). The 12 mouse IL-1 $\delta$   $\beta$  strand domains are recited respectively (Table 3) as Leu8-Asp14, Val19-Asn24, Leu27-Gly31, Ile43-Asn48, Ser56-Val62, Gln67-Thr73, Pro77-Glu82, Phe99-Met106, Leu108-Ser114, Phe121-Ser125, Gln130-Thr135, and Gln153-Asp156 of SEQ ID NO: 2; while the 12 mouse IL-1 $\epsilon$   $\beta$  strand domains are recited respectively (Table 3) as Ser13-Asp19, Val24-Asn29, Ile31-Val35, Ile46-Cys51, Asp63-Val69, Ser74-Lys80, Pro85-Gly90, Ser107-Ser114, Thr116-Ser122, Phe129-Cys133, Cys138-Thr143, and Ile157-His160 of SEQ ID NO: 6).--

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Please replace the paragraph on page 40, lines 31-37 and continuing to page 41, lines 1-2, with the following rewritten paragraph:

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$\beta^6$   
--Alignment of the mouse IL-1 $\delta$  and IL-1 $\epsilon$  sequences (using the Met initiation residue as the first amino acid) with other members of the IL-1 family indicates that the  $\beta$  conformations correspond to similar sequences in other IL-1 family members (see Tables 3, 4, and Fig.2A,B). See also, Bazan, et al. (1996) Nature 379:591; Lodi, et al. (1994) Science 263:1762-1766; Sayle and Milner-White (1995) TIBS 20:374-376; and Gronenberg, et al. (1991) Protein Engineering 4:263-269.--

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Please replace the paragraph on page 42, lines 7-14, and replace with the following rewritten paragraph:

$\beta^7$  --The corresponding location in rodent IL-1 $\delta$  or IL-1 $\epsilon$  (between  $\beta$ 4 and  $\beta$ 5) defines a domain that forms a polypeptide loop which is part of a primary binding segment to the IL-1 receptor type (site B in Fig.2A,B). The loop, depicted pictorially in Figure 1A as protruding into the central axis of the mature IL-1 $\delta$  or IL-1 $\epsilon$  protein, is located between arrows 4 and 5). More precisely, the loop is defined for IL-1 $\delta$  by amino residues Pro47-Ala53 of SEQ ID NO: 2 and for IL-1 $\epsilon$  by amino residues Pro50-Glu58 of SEQ ID NO: 6. Accordingly, IL-1 $\delta$  or IL-1 $\epsilon$  antagonist activity should be generated by removal all or an appropriate portion of a corresponding portion of amino acids located between  $\beta$ 4 and  $\beta$ 5. This suggests that analogous modifications to the loop between the  $\beta$ 4 and the  $\beta$ 5 strands will lead to variants with predictable biological activities. With mouse IL-1RA, it was shown that replacement of the mouse IL-1RA residues with those mouse IL-1 $\beta$  residues introduced IL-1 activity to the IL-1RA variant (IL-1RA could then bind type III receptor). Similar substitutions will establish that type III receptor can probably be used by mouse IL-1 $\delta$  or IL-1 $\epsilon$  proteins or muteins. Additional site B contacts are defined in rodent IL-1 $\delta$  by amino residues 8-11, 13, 112, 114-117, 158 and 160 of SEQ ID NO: 2. Corresponding additional site B contacts are defined in mouse IL-1 $\epsilon$  by amino residues 3-6, 8, 104, 106-109, 154 and 156 of SEQ ID NO: 6. Corresponding residues should be important in the primate sequence (see SEQ ID NO: 13 and 15).--

Please replace the paragraph on page 42, lines 35-36 and continuing to page 43, lines 1-10, and replace with the following rewritten paragraph:

$\beta^8$  --Sites A and C (see Fig. 2A,B) mediate binding of IL-1 $\delta$  or IL-1 $\epsilon$  to the first IL-1 receptor subunit, e.g., an alpha receptor subunit. Site A contacts correspond in IL-1 $\delta$  to amino residues 13-16, 22-24, 29, 31-37, 39, 126-131, 151, and 153 of SEQ ID NO: 2; while site C contacts correspond in IL-1 $\delta$  to amino residues 74-98 of SEQ ID NO: 2. Site A contacts are defined in IL-1 $\epsilon$  by amino residues 18-21, 21-29, 33, 35-